

A. A protein was eluted from a chromatography column with a 1 M NaCl solution. The protein concentration in this fraction is roughly 10 mM. You are trying to reduce the salt content of your protein solution by bag dialysis against 0.1 M NaCl. Assume that the dialysis membrane is permeable for salt ions and water but not for protein. Estimate the initial osmotic pressure at 25°C. State your assumptions.

Assumptions: (5)

- All γ 's are 1
- Temperature is 298 K ~~25°C~~
- 1 M NaCl can be treated as ideal solution
- Protein will not contribute significantly to osmotic pressure because much too dilute (compared to salt)

$$\pi_{in} - \pi_{out} = RT (c_{in} - c_{out})$$

$$\pi = \pi_{in} - \pi_{out}$$

$$\pi = 8.314 \frac{J}{mol \cdot K} \cdot 298 K \left(2 \frac{mol}{L} - 0.2 \frac{mol}{L} \right) \cdot \frac{1 atm}{1.013 \cdot 10^5 \frac{N}{m^2} \cdot 10^{-3} \frac{m^3}{L}}$$

$$\pi = 44.02 atm = \pi_{initial}$$

As you do the experiment, you observe that the volume of your protein solution in the bag increases rapidly. You are afraid that the bag might burst, although you made your bag large enough to easily hold 150 ml. Assuming that the dialysis membrane is only permeable to water but not to salt, what will be the maximum volume of your protein solution at equilibrium? Initially you started with 10 ml solution. You can assume that the NaCl concentration on the outside does not change during the experiment. Ignore any pressure that the bag might exert on the solution. State your assumptions.

Assuming that salt concentration on the outside stays constant and that the protein does not significantly contribute to the osmotic pressure, inside and outside salt concentration can reach the value of 0.1 M

Initially:

$$\text{Inside: } 1 M NaCl \cdot 10 ml = 10 \cdot 10^{-3} \text{ moles } NaCl$$

Finally:

$$0.1 M NaCl \Rightarrow V_{final/max} = \frac{10 \cdot 10^{-3} \text{ moles } NaCl}{0.1 \frac{mol}{L}} = 0.1 L = 100 ml = V_{max}$$

D. For a protein called T4 lysozyme the gene sequence was modified so that two large, hydrophobic amino acids were replaced with the much smaller residue alanine. It was observed that a hole was left in the hydrophobic interior of the protein. When the researchers added benzene to the solution, they found that one benzene molecule did bind in the hole, and made the protein more stable. They know that benzene is slightly soluble in water (2.2×10^{-2} moles/liter in a saturated solution at 25°C). What is the ΔG° for converting one mole of pure benzene to one mole of benzene dissolved in water?

$$\Delta G^\circ = -RT \ln K = -RT \ln \frac{a_{\text{benzene in H}_2\text{O}}}{a_{\text{benzene in benzene}}} = -RT \ln \frac{2.2 \times 10^{-2}}{1 \text{ M}} \quad \text{S}$$

$$\Delta G^\circ = -8.314 \frac{\text{J}}{\text{K mole}} \cdot 298 \text{ K} \cdot \ln 2.2 \cdot 10^{-2}$$

$$\Delta G^\circ = + 9.46 \text{ kJ/mole} \quad \text{S}$$

As expected, transferring one mole of benzene from pure benzene to water is a non-spontaneous ($\Delta G^\circ > 0$) process.

(This refers to a standard state based on concentrations of 1M)

Make an estimate for the free energy of binding of benzene to the protein based on these data.

Explain your reasoning. The hydrophobic interior of the protein can be considered to mimic pure benzene. Binding of one benzene molecule to ~~the~~ fill the hole in the protein, can therefore be considered a transfer process of the benzene molecule from an aqueous phase (protein solution) to a benzene-like phase (interior of the protein). The free energy of binding will therefore be comparable to $\Delta G^\circ = -9.46 \text{ kJ/mole}$. 2

\Rightarrow correlates to a $K_{\text{dissociation}}$ of $2.2 \cdot 10^{-4} \text{ M}$ 5

It was found that the dissociation constant for the benzene from the protein was $4 \times 10^{-4} \text{ M}$.

Does this agree with your estimate? $\Delta G^\circ = -8.314 \frac{\text{J}}{\text{mole K}} \cdot 298 \text{ K} \ln(4 \cdot 10^{-4})$

For Dissociation: $\Delta G^\circ = -RT \ln K_{\text{Diss}} \Rightarrow \Delta G^\circ = -13.4 \text{ kJ/mole}$ for the free energy of binding! 5

$$\Delta G^\circ = +13.4 \text{ kJ/mole}$$

The sign of the free energy of binding estimates agrees. Benzene binds, however, ~~less~~ more tightly to the protein interior than would be expected from the estimate based on the phase transfer model. 3